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(54) Title: MINIFILTRATION METHOD AND APPARATUS

(57) Abstract

An apparatus and related system for small scale processing of solutions. A preferred apparatus is provided in the form of a disposable tip comprising a polymeric housing having a rigid wall portion forming an internal passageway having a longitudinal axis, and a depth filter sealably positioned within the internal passageway. Optionally, the tip provides means for sealably attaching the tip to other devices, either directly or by means of suitable adaptors, at either or both ends of the passageway. The filtration tip can be used in a system of the invention for small scale incubation of samples, e.g., in the course of an assay, reaction, synthesis, binding, extraction, clarification, concentration and the like. An apparatus that includes a hydrophobic support material finds particular use in a method for the preparation of purified oligonucleotides.

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MINIFILTRATION METHOD AND APPARATUS

TECHNICAL FIELD

The present invention relates to laboratory devices for filtering small volumes of sample solutions, and to systems employing such devices. In another aspect, the invention relates to laboratory systems for processing (e.g., incubating, reacting, filtering, and/or evaporating) small volumes of sample solutions.

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of MINIFILTRATION METHOD AND APPARATUS, Serial No. 08/588,727 filed January 19, 1996, which is a continuation-in-part of U.S. application for EVAPORATION FILTER AND METHOD, Serial No. 08/279,444, filed July 25, 1994, which in turn, is a continuation-in-part of APPARATUS FOR RAPID EVAPORATION OF AQUEOUS SOLUTIONS, Serial No. 08/209,786, filed March 11, 1994.

BACKGROUND OF THE INVENTION

The development of filtration devices and methods has burgeoned in recent decades, particularly in the area of bench-top scale processing of solutions containing biomolecule samples.

Such devices and methods, however, are typically used for the filtration of relatively large samples (e.g., on the order of several milliliters and larger). Such systems can rely on any of a number of forces to cause a solution to flow into and/or through the filter, including the use of gravity, capillary flow (e.g., wicking), centrifugal force, fluid or air pressure, and vacuum. Yet, there are comparatively few devices or methods presently available for filtering solutions on a small scale (e.g., less than one milliliter sample size, and particularly less than about 100 microliters).

The small scale filter devices that do exist tend to fall into the general categories of either syringe-type filter assemblies or filter-containing

microcentrifuge tubes. Millipore Corp., for instance, sells an "Ultrafree" brand centrifugal filter device for use with sample volumes between 150 microliters to 15 ml. The device incorporates a high flux ultrafiltration membrane, and is used for concentrating, desalting or purifying aqueous biological samples.

In a related aspect, the use of columns for the synthesis of oligonucleotides has evolved into common use over recent years. Such columns typically contain a support material, such as controlled pore glass, and are capable of being attached (e.g., threaded) to the various devices involved in DNA synthesis and recovery. See, e.g., "Oligo 1000 DNA Synthesizer" Bulletin No. 7910, Beckman Instruments, Inc.

Applicant is unaware, however, of any commercially available devices that can be used to conveniently and simultaneously filter large numbers of small volume samples, particularly in a manner that also allows the device to be used as both a reaction vessel and/or for evaporating the filtrate.

On a separate subject, Applicant's copending application for APPARATUS FOR RAPID EVAPORATION OF AQUEOUS SOLUTIONS, Serial No. 08/209,786, filed March 11, 1994 describes a simple, convenient and inexpensive apparatus for the concentration or evaporation of aqueous solutions typically used in biomedical research and development. In one embodiment the apparatus provides a sample chamber having a plurality of fluid receptacle positions. Atop the chamber is a cover dimensioned to form a substantially air tight seal when in a closed positioned upon the chamber. The cover, in turn, provides a plurality of access apertures. The apertures are each dimensioned to receive a respective air channeling device and positioned to lay in an overlapping position with a respective well position. Also included is a vacuum circuit for drawing a vacuum within the chamber. The vacuum, in turn, causes air or other desired gas to be correspondingly drawn through the air channeling devices and directed toward the vial positions below in order to provide a blow-down evaporative effect.

In another aspect, Applicant's copending US application for EVAPORATION FILTER AND METHOD, Serial No. 08/279,444, filed July 25,

1994 provides a system that involves the use of an evaporation tube having inserted therein a filter that is capable of allowing flow through of the evaporated solvent while substantially preventing the flow through of vaporized analyte material. Also described is an embodiment in which the tube is used within an evaporation unit, such as described above in the previous application. In turn, the latter application describes an embodiment in which a pipet tip, which itself may contain an internal filter, is employed to provide a vacuum blow down effect.

BRIEF DESCRIPTION OF THE DRAWING

In the Drawing:

Figure 1 shows a preferred device of the present invention, in the form of a conical filtration tip.

Figures 2 shows an alternative preferred shape for the filtration tip of the present invention, for use in attaching the tip to a conventional DNA synthesizer.

Figure 3 shows a preferred system of the invention, including filtration tips as shown in Figure 1, each positioned within a respective cover aperture of a vacuum chamber.

SUMMARY OF THE INVENTION

The present invention provides an apparatus suitable for small scale (e.g., on the order of milliliters and smaller) processing of solutions. The apparatus can be inexpensively provided in prepackaged, sterile, and either disassembled or assembled form. In a preferred embodiment the apparatus is provided in the form of a disposable filtration tip comprising a polymeric housing having a rigid wall portion forming an internal passageway having a longitudinal axis, and a depth filter sealably positioned within the internal passageway.

The housing wall provides an internal surface that serves to define the dimensions and shape of the internal passageway. The housing wall also provides an external surface that can be of any suitable shape and dimensions. Preferably, opposing external and internal wall surfaces are generally parallel, and more

preferably, the walls are tapered toward the longitudinal axis along the length of the passageway, in order to form a generally conical tip. Optionally, the tip can provide a passageway that is any other suitable shape, e.g., conical, cylindrical, barrel-shaped, stepped, toric, or frustroconical.

Preferably, the tip further provides one or more means for sealably attaching the tip to other devices, either directly or by means of suitable adaptors, at either or both ends of the passageway. Preferably, the other devices are themselves used to deliver and/or withdraw materials (e.g., solutions) to the filtration tip, and/or to provide positive or negative pressure at either or both ends of the passageway. Optionally, the external surface of the housing can itself be suitably configured to provide attachment means, e.g., for use in press-fitting a conical housing within the aperture of an evaporation block.

The internal filter can itself be of any desirable type, and can include the use of combination filters (e.g., a depth filter in combination with one or more disc filters or other depth filters), and filter assemblies (e.g., in which the depth filter serves to retain other useful materials, such as particulate materials). The internal filter is preferably positioned within the passageway of the tip in order prevent the free flow of solution through the tip, and to provide a filter-free inlet passageway capable of retaining the solution to be filtered. Preferably, the filter position also provides a filter-free outlet passageway. Suitable tips, and filters, provide an optimal combination of such properties as wettability, porosity, stability, activity (or inertness) and cost.

The invention further provides a system for processing solutions, one embodiment of the system comprising:

(a) a vacuum chamber dimensioned to sealably enclose one or more receptacle sites, the chamber comprising a cover portion comprising one or more apertures, each in an overlapping position with respect to a corresponding receptacle site,

(b) one or more filtration tips in a sealed relationship with the cover, each with its outlet passageway directed toward a respective receptacle site, and

(c) a vacuum system for controllably creating a vacuum within the chamber in a manner sufficient to draw solution from the inlet passageway of a filtration tip, through the filter and into a corresponding receptacle site.

Any or all of the component parts of a system of the present invention can be provided as integrally molded pieces, in order to facilitate their use and provide the desired sealed relationship. For instance, the cover portion can be molded so as to provide one or more tip-like portions in integral form.

In a related aspect, the invention provides a method of processing a sample, the method comprising the steps of:

(a) providing a system comprising a vacuum chamber and one or more filtration tips as described above;

(b) delivering solution to the inlet passageway of the filtration tip(s);

(c) optionally, using the inlet passageway as an incubation vessel for the solution;

(d) creating a vacuum in the chamber in order to draw the solution into the filter;

(e) optionally, using the filter as an incubation vessel for the solution;

(f) employing vacuum in the chamber in order to draw the solution through the filter and into a receptacle site, and optionally;

(g) employing the vacuum, with optional heating, to create a vacuum blow down effect in order to evaporate the filtrate.

The final product can be recovered and used in a conventional fashion, such as by resuspending, concentrating, or other further processing steps. As can be seen, the ability to control the vacuum, and in turn control the flow of solution into and/or through the tip, provides the opportunity to use the filtration tip as an incubation vessel. The inlet passageway, for instance, can itself be used as a site to incubate solution in the course of an assay, reaction, synthesis, binding, extraction, clarification, concentration and the like.

Similarly, the filter can be used to incubate the solution, including any reaction product of the solution resulting from incubation in the inlet passageway.

Thereafter, final product can be recovered in any suitable fashion, including from the filtrate, or from within the filter itself.

In a particularly preferred embodiment, the apparatus and system can be used to perform a variety of steps involved in the synthesis and recovery of oligonucleotides. In such an application, the system provides an optimal combination of such properties as ease of use, cost, speed, convenience, and recovery.

DETAILED DESCRIPTION

The present invention provides an apparatus suitable for processes that include the small scale filtration of solutions. The apparatus, *per se*, can be provided in the form of a disposable, rigid polymeric tip containing an internal filter. The filtration tip, in turn, can be used in a system of the present invention, the system providing vacuum and/or pressure means for controllably causing a solution to flow into or through the filter. A vacuum system, for instance, comprises a vacuum chamber for controllably drawing solutions through the filtration tip.

Surprisingly, the system provides the user with the ability to simultaneously and/or sequentially perform a variety of processing steps - including incubation, filtration, and evaporation steps - all on a milliliter or smaller scale, using the same device.

The following words, and inflections thereof, will have the meanings ascribed to them when used in reference to a system of this invention. The word "incubation" will refer to any processing step or steps that can be carried out within the solution, e.g., whether within the inlet passageway or within the filter (or components thereof) itself. Examples of such steps include, but are not limited to, those involved in performing an assay, reaction, synthesis, binding, extraction, clarification, concentration and the like. In the synthesis of oligonucleotides, for instance, each of the various synthesis, and subsequent cleavage and deprotection

steps, can be accomplished in the inlet passageway and/or filter of an apparatus of this invention.

The word "filtration", and inflections thereof, will refer to the passage of solution into or through the filter of a tip, including any further processing steps (e.g., incubation, affinity binding, size exclusion, etc.) involved therewith. As used herein with respect to a filtration tip, the word "filter" will refer to one or more discrete materials or assemblies, or combinations thereof, within a tip of the invention. The word "evaporation" will refer to the use of a system of the present invention, e.g., using non-filter containing replacement tips, to evaporate filtrate in order to concentrate or dry a sample.

A filtration tip, as described herein, can be used with a variety of solutions, including aqueous and/or organic solutions. The tip can be used to provide an incubation vessel for use in procedure involving an incubation step, such as a reaction, synthesis, extraction, wash, and/or reconstituting step. The tip can also be used in combination with other devices, including an evaporation system as described below.

In a preferred embodiment, the filter is suitably dimensioned and capable of retaining a solution in the inlet passageway, such that flow (e.g., gravity or capillary flow) of the solution occurs only minimally, if at all, into or through the filter. When used with a vacuum system, the filter is capable of permitting the solution to flow into and/or through the filter upon application of a vacuum to the outlet passageway, which typically extends into an otherwise sealed vacuum chamber.

For instance, the filtration tip can be used to provide a system for processing solutions, the system comprising:

(a) a vacuum chamber dimensioned to retain one or more solution receptacles, the chamber comprising a cover portion comprising one or more apertures, each in an overlapping position with respect to a corresponding receptacle site,

(b) one or more filtration tips, as described herein, each tip being integrally formed with, or sealably positioned within, a respective cover aperture, with its outlet passageway directed toward a receptacle site, and

(c) a vacuum system for controllably creating a vacuum within the chamber that is sufficient to draw solution from the inlet passageway through the filter and into a receptacle site.

Receptacle sites can be provided individually, e.g., a separate receptacle can be provided for each aperture/filtration tip combination. The receptacle sites can also be shared by one or more aperture/filtration tip combinations, e.g., in the form of a single or combined receptacle site, or a trough for collecting unneeded rinse or wash solutions. The receptacle sites can themselves serve to hold the filtrate, e.g., in the manner of a tray of wells. Alternatively, the receptacle sites can be provided in the form of recesses for retaining removable receptacles, such as individual tubes.

Apparatus.

Filtration tips can be of any suitable form and capacity. With reference to the tip shown in Figure 1, it can be seen that an apparatus 10 provides a polymeric housing 12 having a rigid wall portion forming an internal passageway 14 having a longitudinal axis 16, and a filter 18 sealably positioned within the internal passageway.

The housing 12 provides an internal surface 20 that serves to define the dimensions and shape of the internal passageway 14. The housing wall also provides an external surface 22 that can be of any suitable shape and dimensions. In this particular case, the external surface of the housing is itself conical, in order to facilitate its use by press-fitting it into an aperture.

As shown in Figure 1, the tip optionally also provides means 24 for sealably attaching the tip to other devices, including adaptors for other devices, at either or both ends of the passageway. Such other devices can be used to deliver and/or withdraw materials (e.g., solutions) to or from the filtration tip. Examples

of which include pipettors, syringes, columns, and the like, and include the use of an apparatus of the invention as an in-line filter in conjunction with the use of other devices. Such other devices can also be used to provide positive or negative pressure at either or both ends of the passageway. Examples of these devices include syringes, vacuum chambers, and the like.

The internal filter 18 can itself be of any desirable type or combination of types, and can include the use of combination filters, and filter assemblies that serve to retain other useful materials, such as particulate materials. In the embodiment shown in Figure 1, a filter in the form of a single plug-like material is positioned midway within the passageway of the tip in order prevent the free flow of solution through the tip, and preferably, to provide a filter-free inlet passageway 26, for the solution to be filtered, and a filter-free outlet passageway 28, for optional recovery of the filtrate. Suitable tips, and filters, provide an optimal combination of such properties as wettability, porosity, stability, activity (or inertness) and cost.

In Figure 2 there is shown an alternative embodiment of the apparatus of the present invention, which is adapted to be attached to a conventional device for DNA synthesis. As can be seen, the alternative apparatus 30 is formed of a rigid polymeric wall 32 having an outer surface 34 and an inner surface 36 defining a generally cylindrical passageway 38. Within the passageway, however, there can be seen a slightly tapered region 40 within which filter 42 can be press-fit, much in the manner of the apparatus of Figure 1. The apparatus of Figure 2, however, is provided with attachment means (e.g., luer fittings) at both its inlet end 44 and outlet end 46, permitting the apparatus to be attached to the tubing set of a DNA synthesizer. The external surface of the housing is itself generally cylindrical, provided with outer ridge 48 in order to approximate the general configuration of a conventional DNA synthesis column that it is intended to replace.

A preferred tip of the invention generally provide a cylindrical or conical passageway. Preferably, a plug-like depth filter, including a combination filter or a filter/particulate assembly constructed using one or more depth filters, can be

positioned within a conical tip by simply press-fitting the filter to the desired location. Alternatively, the filter can be retained in any suitable manner, e.g., it can be positioned so as to rest on an internal ridge within an internally stepped tip. The term "depth filter", as used herein, will refer to a material having suitable physical properties and dimensions to allow it to be sealably retained (e.g., by press-fitting) within the housing. The word "sealably", as used in this respect, will refer to the ability of a filter to prevent or permit the flow of solutions into or through the filter to a desired extent.

The depth filter can itself provide the desired filtration effect (e.g., reactivity, affinity, size, molecular weight) and/or it can be used to support and/or retain one or more other materials that provide the desired filtration. A depth filter can be used, for instance, to support a disc filter within the housing. Alternatively, a pair of depth filters can be used to retain a particulate material within the housing, e.g., by providing a "sandwich" effect.

Housing.

Typically, the apparatus housing used in the present apparatus will provide a passageway having a nominal volume of between about 10 and 500 microliters, and preferably between about 100 and 300 microliters. The tips themselves are typically, and preferably formed (e.g., molded) of a rigid, polymeric material such as polypropylene or polyethylene, having a smooth, nonwetting surface. Such tips are preferably capable of being sterilized, e.g., by radiation, ethylene oxide, or conventional autoclaving. Beckman Instruments, Packard Instrument Company, and others sell tips in a variety of optional configurations, e.g., for use with automated workstations and robotic liquid handler system.

An internal wall providing a straight, tapered, stepped or beveled transition region can be provided in any suitable manner, and generally provides at least two functions. First, it provides an internal region into which a suitably dimensioned filter can be snugly fit and retained. Also, a sloped or stepped external surface of the tip provides the ability to easily insert and sealably retain the tip in a cover aperture.

Filtration tip housings can be provided individually or in suitable combinations such as strips. Optionally, a chamber cover can itself be provided having one or more pipet tips integrally molded therein. Such a cover can be dimensioned, for instance, to sealably fit a microtiter tray in order to form a two part vacuum chamber. Ideally, the microtiter tray is provided with sides that are specially configured to provide a suitable seal with a cover portion. The tray and other components are also suitably strong, to enable them to withstand the vacuum, pressures and solutions encountered in the course of their use.

For use with a 96 well receptacle, for instance, filtration tips can be provided in a compatible 96 tip configuration. U.S. Patent No. 4,511,534, for instance, describes the use of a "pipet plate" having 96 pipets integrally formed for use in a liquid transfer system. In view of the present description, those skilled in the art will appreciate the manner in which such a pipet plate can be prepared, fitted with filters, and used in the manner presently described.

Preferred filtration tips for use in a conventional 96 well format have a nominal pipetting volume of 200 microliters (e.g., approximately 2" length, with upper diameter of 0.3" O.D., and a lower, tip diameter of 0.05" O.D.). The filter within the tip can be positioned approximately midway into the tip, allowing about 50-100 microliter capacity for solution within the inlet passageway. Alternatively, the material can be provided having a narrower width dimension, and positioned nearer the tip of the pipet tip, to provide an inlet passageway volume of up to almost 200 microliters available for a solution.

In order to fit the 96 well format the tip orifice preferably has an external diameter of not more than about 0.35". If, however, more volume is desired in the inlet passageway, the tip can be extended on upper top portion using a matable cylindrical portion that can accommodate additional volumes (e.g., up to 3 ml or more) of solution.

Filters.

The filter itself is preferably of a suitable type and dimensions to prevent the flow through of sample under atmospheric conditions, and to allow flow to begin upon the application of a vacuum and/or pressure to the tip.

As described above, filter can be provided in a variety of forms, including in the form of a plug that can be press-fit into a pipet tip to form a filtration tip of the invention. Plugs and/or discs having a diameter of on the order of 2-10 mm, and preferably 3-5 mm are particularly useful, and provide a tight seal when press fit into a standard conical pipet tip of 25 microliter to 1000 microliter nominal capacity. Optionally, for instance, a filter assembly can be provided in the form of a solid phase filter material retained within a filtration tip, e.g., between a pair of respective inlet and outlet passageway plugs.

Filters can be inserted in the pipet tips in any suitable manner, e.g., they can be sold separately and inserted at the time of use. They can also be prepackaged as a combination, e.g., singly or in convenient rack packaging that allows one-handed multi-tip access.

Useful filters are described, for instance, in U.S. Patent No. 5,364,595, the disclosure of which is incorporated herein by reference. The '595 patent describes pipet tips having porous plastic plugs, the tips being useful for preventing solutions from escaping as aerosols in the course of pipetting. The plugs have pore sizes suitable to prevent liquid samples from being drawn through the plugs by the suction applied using a conventional suction (i.e., pipettor) device. However, Applicant has found that, using a chamber and system of the present invention, a vacuum can be drawn and controlled that is sufficient to cause the flow of solutions through such filters.

Preferred filters provide an optimal combination of such properties as porosity, hydrophobicity/hydrophilicity, throughput, swellability, inertness and cost. Typical disc-type filters, for use in combination with a depth filter, have a porosity of on the order of 0.2 microns (for sterile filtration) to 0.45 microns (e.g., for fine particle and microbial filtration). Depth filters, in turn, typically

have a larger porosity, e.g., on the order of about 1 micron to 100 microns, and preferably between about 2 microns and about 20 microns. Plug-like materials, such as those used for supporting disc filters, are generally sufficiently porous so as to not negatively affect the desired flow characteristics of the disc itself.

Filters can be also prepared from a number of other natural or synthetic materials, from organic polymers, inorganic polymers, ceramics, and metals, and combinations thereof. Where the material is an organic polymer, the filter can be prepared from a polyurethane, polyacrylonitrile, polyvinyl alcohol, polyvinyl acetate, polyester, polyamide, polystyrene, polysulfone, cellulose, cellulose acetate, polyethylene, polypropylene, polyvinyl fluoride, polyvinylidene fluoride, polytrifluorochloroethylene, polyvinylidene fluoride-tetrafluoroethylene copolymer, polyethersulfone, poly(meth)acrylate, butadiene-acrylonitrile copolymer, polyether-polyamide block copolymer, and ethylene-vinyl alcohol copolymer.

Preferred filters are capable of being extracted, in order to recover biomaterial that might come into contact with the material. Preferred materials are also stable to sterilization, such as by gamma radiation, ethylene oxide, and heat sterilization.

The filter in the tip can be of any suitable material for performing filtration and selective removal of molecules from a solution. Typical applications include particle filtration that allow a filter of a wide variety of pore sizes to more specific filtration such as sterile filtration where pore size is below 0.2 micron size. Filter of special properties for removal of proteins, nucleic acids, and DNA preparations including plasmids for sequencing, genomic DNA, mini-scale and large scale preparations of DNA.

Preferably, filters are inert (i.e., have no reactivity other than that intended), sterile, and capable of being certified RNAse/DNAse free. Materials and tips can be provided in any desirable color, although tips are preferably clear in order to more easily see fluid levels.

When used for incubating solutions, e.g., as a support for the synthesis of oligonucleotide, a filter is preferably provided in the form of a sintered body

comprising both a matrix component (e.g., in the form of a porous thermoplastic matrix such as polyethylene, polypropylene or PTFE) and a resin component (e.g., such as CPG, polystyrene, or the like). See, for instance, EP Application No. 659482 (Giordano et al.), the disclosure of which is incorporated herein by reference.

Optionally, such resin components can be compounded with a suitable lipophilic support material such as C18-silica, for use in purification (e.g., of DMT-DNA), solid phase extraction (e.g., of organic molecules), and other applications. Resin can be mixed with matrix material in order to provide a useful amount of resin activity (e.g., on the order of 5 nmole or greater). Additional activity can be provided, if desired, by the use of additional filter and/or by functionalization of the material itself.

A sintered body can be prepared and suitably dimensioned, e.g., by conventional die-cut processes, and press-fit into a pipet tip to form a filtration device. The apparatus, in turn, can be fitted with appropriate attachment means (e.g., top and bottom male and female luer adaptors) in order to facilitate its use in conventional DNA synthesizers.

Filters can themselves be reactive, or can contain reactive components (e.g., particles). Examples of preferred reactive filters include, but are not limited to, the "Empore" brand products available from 3M. Empore brand products include those used as extraction discs, or for extracting organic compounds from water sources and pollutants from ground water. Also included are Empore brand extraction membranes, for extracting plasmid DNA from cell lysate in the course of sequencing analysis and other uses.

Systems.

The filtration tips and system of the invention can be used to accomplish a variety of steps in the course of processing samples such as those containing synthetic biomolecules. For instance, the tips can be used as an incubation vessel, filtration means, and/or for evaporating samples within the receptacle. Filters can themselves be reactive, e.g., functionalized or derivatized in order to perform

desired reactive or binding functions. Processes such as reaction, filtration, and evaporation can be performed individually or sequentially, in order to provide an efficient, convenient, cost effective, and simple means for processing samples.

The system also facilitates the simultaneous processing of several samples. The chamber can be configured, for instance, to retain a one or more receptacle sites, e.g., including sites provided in the form of a standard 96 well microtiter plate. Using a cover portion having 96 corresponding apertures, and in turn providing 96 filtration tips, the system can be readily integrated with robotic and automated liquid handling means already available for the 96 well format. The ability to simultaneously and sequentially perform the steps necessary to process 96 different samples, from start to finish, provides a particularly powerful and versatile tool, especially considering the convenience and low cost of such a system.

Preferably, the system employs a chamber such as described in Applicant's copending application Serial No. 08/209,786, filed March 11, 1994. In one embodiment, as can be seen in Figure 3, the system 60 comprises a block 62 having a top surface 64 comprising a sample chamber 66, the chamber comprising a plurality of well positions 68 dimensioned to releasably hold sample vials 69 in an upright, stable retained position within the chamber. Covering the chamber is a cover 70 dimensioned to form a substantially air tight seal when in a closed positioned upon the chamber.

The cover provides a plurality of access apertures 72, each provided with a recessed portion of greater circumference 73 into which a rubber septum 75 can be placed when not in use. Aperture 72 itself traverses the thickness of the cover and is dimensioned to receive a respective filtration tip apparatus 10 (shown prior to placement in the aperture). Apparatus 10 can be inserted into the aperture (with the septum removed) and positioned to lay in an overlapping position with a respective well position when the cover is positioned upon the chamber. Also included is a vacuum circuit 74 for drawing a vacuum within the chamber. The vacuum, in turn, causes air or other desired gas to be correspondingly drawn

through the tips and directed toward the vial positions below in order to provide a blow-down evaporative effect.

Optionally, the system also includes the use of a receptacle tube fitted within a filter, e.g., in the manner described in Applicant's copending US application Serial No. 08/279,444. The system employs a tube having a filter capable of allowing flow through of evaporated solvent while substantially preventing the flow through of vaporized analyte material.

Use of an Apparatus and System.

In a related aspect, the invention provides a method of processing a sample, the method comprising the steps of:

(a) providing a system comprising a vacuum chamber and one or more filtration tips as described above;

(b) delivering a solution to the inlet passageway of the filtration tip(s);

(c) optionally, incubating the solution within the inlet passageway;

(d) creating a vacuum in the chamber in order to draw the solution through the filter and into a receptacle site, and optionally,

(e) employing the vacuum, with optional heating, to create a vacuum blow down effect in order to evaporate the sample contained in the receptacle.

Solutions can be delivered to the inlet passageway of the filtration tip(s) in any suitable manner. In one embodiment the pipet can be fitted, via an adaptor, with a syringe or other suitable solution reservoir. An alternative embodiment of the system of the invention therefore comprises the use of a delivery device, such as a syringe, that can be sealably connected to a filtration tip at its inlet passageway. A syringe can be used as a reservoir for solutions, or optionally, a second filter (e.g., disc-type) can be interspersed between the syringe and the filtration tip. Examples of suitable syringe filters, and luer lock connectors, are available from a number of commercial sources, such as Nalgene.

Once in place within the inlet passageway, the solution can optionally be incubated, for instance, by causing or allowing a reaction, synthesis, binding, extraction, clarification, concentration or other processing step to occur. Upon the

completion of incubation, or at any other appropriate time, the vacuum circuit can be employed in order to draw the solution into and/or through the filter.

The solution can also be drawn into the filter, that is, without being drawn entirely through it. Within the filter, the solution can again be subjected to an incubation step, either while the solution remains static in the filter, or as it is slowly drawn through it. In this embodiment, the filter can serve the purpose of a solid support, e.g., a chromatographic, affinity, or other support.

Optionally, and preferably, the vacuum circuit is employed in order to draw the solution fully through the filter and into a receptacle site. Otherwise, the filter can either be used *in situ*, or can be removed and transferred to another container where it can undergo further processing.

Once the filtrate is drawn into a receptacle site, the vacuum circuit can be employed, with optional heating, to provide blow down effect in order to evaporate (e.g., concentrate or dry) the filtrate. The blow-down step can be accomplished using the same filtration tip, or another tip (with or without filter) in place of the filtration tip.

In an optional embodiment, as referred to above, means can be employed to provide a temperature that is either elevated or reduced as compared to ambient conditions. For instance, the vacuum chamber can be provided in a form that allows it to be placed in a water bath at an elevated temperature in order to facilitate evaporation.

Oligonucleotide Synthesis.

With respect to the synthesis and recovery of oligonucleotides, the system can be used in the following manner to accomplish a wide variety of steps involved in the synthesis, cleavage, deprotection, and recovery of the final product;

(a) using the inlet passageway as an incubation vessel (e.g., for synthesizing oligonucleotide and/or cleaving or deprotecting the oligonucleotides from a solid support material placed atop the filter);

(b) creating a vacuum in order to draw solutions through the filter and into a solution receptacle within the chamber, leaving the solid support atop the filter;

(c) evaporating the solution from the cleaved oligonucleotide by vacuum blow-down, and

(d) optionally, resuspending the final product in the solution receptacle.

In yet a further optional embodiment, reagents and solutions can be applied in the gas phase, in the manner described in copending application US Serial No. 08/230,766, filed April 21, 1994. In such an embodiment, the method comprises the step of incubating the solid support in an environment comprising gaseous cleavage/deprotection reagent. Preferably, the newly synthesized oligonucleotide is the synthetic product of a cyanoethyl phosphoramidite process and is protected by a labile protecting group. In such case, the gaseous cleavage/deprotection reagent is selected from the group consisting of gaseous ammonia, gaseous methyl amine, ammonium hydroxide vapors, and mixtures thereof.

In use, the system of the present invention can be used with an apparatus for recovering synthesized oligonucleotides from a solid support, the apparatus comprising (a) a sealable chamber for incubating the solid support in an environment comprising gaseous cleavage/deprotection reagent, (b) a supply of cleavage/deprotection reagent capable of being delivered to the chamber in a gaseous form, and (c) a gas delivery conduit for delivering the reagent to the chamber in a gaseous form in order to cleave and deprotect the oligonucleotides.

In yet a further preferred embodiment, an apparatus and related compositions of the present invention can be used for synthesis of oligonucleotides as well as for cleavage, deprotection and purification of the newly synthesized oligonucleotides. In a particularly preferred embodiment, each of the various steps involved in synthesis and purification are performed in the same column and include the use of reagents provided in the gas phase, optionally in combination

with reagents provided in the solution phase, in a manner that obviates the need to remove and/or reapply the newly synthesized oligonucleotides to the column.

Conventional synthetic approaches are described in T. Brown and D. Brown, "Modern Machine-aided Methods of Oligonucleotide Synthesis", Chapter 1, pp.1-24 in Oligonucleotides and Analogues: A Practical Approach, F. Eckstein, ed., Oxford University Press 1991, the disclosure of which is incorporated herein by reference. Briefly put, an oligonucleotide is synthesized by adding one nucleotide monomer at a time, with the first nucleotide being attached by a linker arm at its 3'-terminus to a solid support. The solid support can generally be CPG, polystyrene, or any of a number of other supports designed for that purpose.

A solution containing an excess of a second nucleotide monomer is added, the monomer having been activated at its 3'-phosphate position to facilitate condensation, and protected at its 5'-hydroxyl terminus (by a dimethoxytrityl ("DMT") group) to prevent self-polymerization. The 3'-terminus of the free nucleotide is attached to the 5'-hydroxyl of the growing chain by the formation of a phosphate triester. The phosphate triester is then oxidized to a stable phosphotriester before the next synthesis step.

After each round of monomer addition, excess reagents are washed off the column, and any unreacted 5'-hydroxyl groups are blocked or "capped" using acetic anhydride. While this step has the advantage of preventing further monomer additions, it also means that the corresponding oligomer will be incomplete, truncated at the point of being capped. The 5'-DMT group of other bound oligomers will then be removed using 80% acetic acid to allow those oligomers to react with another activated monomer in the next round of synthesis.

Finally, the fully assembled oligonucleotides are cleaved from the solid support and deprotected, generally to be purified by HPLC or some other method. Applicant's U.S. Patent No. 5,514,789 describes the use of cleavage/deprotection reagents in the gaseous phase. The use of such reagents permits the cleaved, deprotected oligonucleotide to remain in position upon the original support.

It can be seen therefor, that absent HPLC or other post-synthesis purification, the result of a conventional oligonucleotide synthesis will include a heterogeneous population of oligonucleotide lengths, with a fair percentage of oligonucleotides having been undesirably truncated short of the desired length. Although it is possible to purify the oligonucleotide preparation to recover only those of the desired length, in view of the added costs and difficulty involved, the oligonucleotide preparations are often sold as the original mixture, which can contain on the order of 20-40% truncated oligonucleotides.

The DMT protecting groups referred to above provide an optional means for purifying oligonucleotides, since only oligonucleotides of the full intended length should possess a terminal DMT group. DMT purification typically involves the following steps: (1) the cleaved, deprotected (but still DMT-protected) oligonucleotide mixture is eluted from the original support material, and combined (or re-combined) with a suitable hydrophobic support, such as polystyrene, having affinity for the lipophilic DMT group; (2) since any truncated (i.e., failure) sequences present in the preparation will be lacking a terminal DMT group, those oligonucleotides will not attach to the support and can be removed by washing the support with 3% ammonium hydroxide; (3) the oligonucleotides that remain attached to the support are then fully deprotected (i.e., detritylated by removing the DMT groups) by the use of a reagent such as trifluoracetic acid, and finally, (4) the purified and fully deprotected oligonucleotide preparation is eluted from the support.

Cartridges are manufactured and sold for the sole purpose of performing DMT purification, e.g., polystyrene columns sold under the tradename "OPC" sold by Applied Biosystems, Inc. Such cartridges must generally be conditioned, e.g., by the use of acetonitrile and triethylammonium acetate, prior to use.

Other polystyrene-based supports have been manufactured and sold for use as synthetic supports in oligonucleotide synthesis. U.S. Patent No. 5,262,530, for instance, describes a method for synthesizing a polynucleotide immobilized on a nonswellable porous polystyrene support in the form of a rigid 10-60% crosslinked

polystyrene support. The patent describes a solution phase synthetic process, followed by a solution phase cleavage/deprotection regimen that results in solubilization of the oligonucleotides and removal of the oligonucleotides from contact with the support. In order to purify the resultant oligonucleotide preparation, as described above, the oligonucleotides can either be subjected to HPLC or reapplied to a suitable hydrophobic support in order to perform DMT purification.

Applicant has discovered that, by the use of gas phase reagents, suitable hydrophobic (e.g., polystyrene) support materials can be used for synthesis and/or DMT purification of the resultant oligonucleotides, all in the same column and without the need to elute and/or reapply the oligonucleotides to or from the column. In a preferred embodiment, that column is a minifiltration column of the present invention, having support material contained within or between one or more depth filters.

Applicant has previously achieved and described a similar result (namely, synthesis, cleavage/deprotection and DMT purification, all in a single column) through different means, as described in Applicant's copending US Application Serial No. 08/755,398. That application provides a rapid, solution phase method of cleavage, deprotection, desalting and/or recovery, each of which can be performed in the same reaction vessel, and optionally, while on the synthesizer itself.

In the present application, Applicant provides an approach based on the unique combination of gas phase reagents and hydrophobic supports. Suitable support materials for use in the present invention provide both a support function for oligonucleotide synthesis and a hydrophobic function for use in DMT purification. Those functions can be provided by the same or different components of a support material. Suitable support materials include polystyrene and other materials described in U.S. Patent No. 5,262,530 (the disclosure of which is incorporated herein by reference) and suitable combinations of polystyrene with conventional materials such as CPG.

In a particularly preferred embodiment, the support material is a combination of a functionalized CPG diluted with a non-functionalized polystyrene support component. The functionalized CPG serves as the support during the synthesis of the oligonucleotide. Following synthesis, cleavage and deprotection is performed using gas phase reagents, allowing the cleaved, deprotected oligonucleotide preparation to be retained on the column. The hydrophobic component then serves its major role in the course of DMT purification as described below.

In the present invention the solid support material can be provided in any suitable format, e.g., in the form of a minifiltration tip, conventional synthesis column, or the wells of a 96 well microtiter plate. In each embodiment, in view of the buoyancy of supports such as polystyrene it is preferred that the support be physically retained within the column, as by depth filters and the like. Each well of a 96 well microtiter plate can be fitted, e.g., with a frit or a depth filter at the bottom of the plate, in order to retain a loose support, e.g., in the form of a membrane or particulate material.

A depth filter, in turn, can itself be functionalized, for instance, with nucleoside. Multiple sample processing using such microplates can also be performed using regular scale synthesis, e.g., in the range of about 30 nmol to about 200 nmol of product per well. The process will thus provide for the convenient cleavage and deprotection of up to 96 samples, in such amount per plate.

In a preferred embodiment, an oligonucleotide preparation is first synthesized on a support contained within a minifiltration tip of the present invention using a conventional solution phase approach described above. The synthesized oligonucleotides are then cleaved and deprotected using reagents provided in the gaseous phase (as described in U.S. Patent No. 5,514,789). The cleavage/deprotection reagent (generally an amine reagent) is then removed by suitable means, e.g., by flushing the column with air or organic solvents.

If the oligonucleotide has been synthesized without the use of DMT protecting groups, it is possible to proceed directly to the use of an organic solvent (e.g., acetonitrile) in order to remove organics (in effect "desalt" the support), while permitting the oligonucleotides to remain on the support. Optionally, residual solvent can then be removed prior to eluting the oligonucleotide product with water.

In a preferred embodiment the newly synthesized oligonucleotide includes the use of DMT-protecting groups. Following cleavage and deprotection in the gas phase the failure sequences can be removed by the use of a solvent having suitable properties (e.g., polarity) to permit the DMT to remain bound to the support while again desalting the support by removing organics. Examples of such solvents include 1% to 5% ammonium hydroxide, and 1% to 5% acetonitrile in triethylammonium acetate. Without being bound by theory, the DMT-protected oligonucleotides appear to remain on the support by virtue of (1) the affinity of the DMT groups for the lipophilic (e.g., polystyrene) component of the support and (2) their presence as a salt (e.g., ammonium salt) that is insoluble in organic solvents such as acetonitrile. Prior to removing failure sequences the support can optionally be conditioned by suitable means, e.g., by the conventional means of treatment with aqueous acetonitrile (e.g., 1-5%). Residual acetonitrile can be removed by flushing the column with air, or by other suitable means. As another optional step, the column and its contents can then be further conditioned, e.g, by treatment with triethylammonium acetate.

Following desalting and/or conditioning, and the removal of failure sequences the oligonucleotides that remain on the column (presumably DMT-protected oligonucleotides of a constant, desired length) can be detritylated by suitable means, typically a suitable acid such as 1-2% trifluoroacetic acid. The acid is washed off with water and the purified, detritylated oligonucleotide preparation can be eluted from the column (e.g., with a stronger solvent such as 5% to 20% aqueous acetonitrile), or used in situ.

The invention will be further described with reference to the following non-limiting Examples.

EXAMPLES

Example 1

Flow Characteristics

The flow characteristics of solutions were evaluated using aerosol filter tips in an evaporation block having an 8 vial capacity.

Tips (referred to herein as "A" tips) were provided having a polyethylene depth filter positioned midway into a standard 200 microliter pipet tip. The filter itself was about 2 mm in diameter and 4 mm long (Midwest Scientific, Product No. AN-20FB "Tip Sentry" pipet tips). Other tips ("B" tips) were provided having a polyethylene filter positioned at the upper portion of a regular 200 microliter pipet tip. The B tip dimensions were about 3 mm in diameter and 3 mm long (Midwest Scientific, Product No. AN-100FB).

An evaporation block having an 8 vial capacity was fitted with 8 type A tips positioned through the apertures in the lid. The vacuum was adjusted to 5" Hg using a clamp, using a laboratory diaphragm pump as the vacuum source. Using a syringe filled with water, water was added to the inlet passageway of the pipet tip. It was found that seven drops would flow through the tip in 30 seconds. At 10" Hg it was found that 30 drops of water would flow through the tip in 30 seconds. At 5" Hg 16 drops of isopropanol would flow through the tip in 30 seconds and at 1 0"Hg the flow was 32 drops in 30 seconds. At lower vacuum the difference in flow rate was more pronounced as compared with the flow at higher vacuum.

This Example demonstrates that the vacuum drawn by a standard laboratory pump is sufficient to controllably pull solutions through the filtration materials used.

Example 2

Aqueous/Organic Solutions

At a vacuum of 23" Hg, using filtration tip A, 2 ml of water was found to pass through the tip in 70 seconds. With isopropanol, 2 ml was able to pass through the tip in 120 seconds. This experiment shows at high vacuum there is a difference in flow rate between an organic solvent and water.

Example 3

Effect of Filtration Material Surface Area

Using a filtration tip identified as type B above, the following experiment was performed using the protocol described above.

2 ml of isopropanol was found to flow through the filter at 23" Hg within about 90 seconds, with a corresponding water flow rate of about 50 seconds for the same volume. These results demonstrate that flow rate can be increased by the use of filters having larger diameter (and in this case a larger surface area as well).

Example 4

Filter Assembly

An evaporation block as described above was fitted and used with a nylon membrane filter in the following manner. A standard 200 microliter pipet tip was fitted with a male to male luer connector (Qosina Corp. New York, New York). A female to female luer connector (Value Plastic, Fort Collins, Colorado) was fitted on top of the male connector, leaving a female port for a disk filter (Spartan®-3 filter, Schleicher and Schuell, Keene, New Hampshire, Nylon membrane, 3 mm, pore size 0.45 micron). All 8 apertures within the cover of the unit were fitted with the nylon membrane assembly and used to evaluate the flow rate of water through the filter, having a defined pore size.

It was found that at 23" Hg, 2 ml of water was able to flow through the filter in 45 seconds. It was noted that the flow rate through a 3 mm nylon filter of 0.45 micron was about the same rate as the tip type B, in Example 3. The porosity of the filter in the pipet tip would thus indicate to be in the same pore size range as the defined nylon filter. This observation is also valid when assessing the feasibility to use other filter in the pipet tip, i.e. material that is commonly used in disk filter of the type commercially available.

Example 5

Oligonucleotide Recovery

In the course of synthetic oligonucleotide synthesis, the completed oligonucleotide typically needs to be cleaved from its bound position on a support, and deprotected in order to be recovered and used. The oligonucleotide is typically cleaved from the support using ammonium hydroxide, e.g., by incubation for 60 to 90 minutes at room temperature. Thereafter, the cleaved oligonucleotide can be heated in the same solution, e.g., at 55°C for 10 hours, to complete the deprotection of the side chain protecting groups.

Alternatively the cleavage and deprotection steps can be performed on the support itself, and within a vial containing ammonium hydroxide. Following cleavage and deprotection, the support materials itself is typically removed form the fully deprotected oligonucleotide in solution. This is typically done by centrifugation, in order to sediment the support material, followed by aspirating or pouring the solution containing the oligonucleotide into another vial, for evaporation.

This Example demonstrates that the filtration tips and system of the present invention can be used to recover such products by filtration and evaporation of the cleaved, deprotected oligonucleotide sample using a single device.

DMT-DNA (18 nucleotides in length) at 0.2 micromolar scale are synthesized on CPG support (500 Angstroms). After the completion of synthesis

the residual acetonitrile in the column is removed by flushing the column with air using an empty 10 ml syringe.

Following synthesis, the support materials is emptied into a vial and filled with ammonium hydroxide. It is then heated at 55°C for 10 hours, in order to cleave and deprotect the oligonucleotide from the support. Rather than centrifuging the sample, however, the support and solution are filtered using an evaporation block having filtration tips within the cover apertures. The filtrate, containing cleaved and deprotected oligo, is collected within vials positioned in the vacuum chamber. The flow through the pipet tip is accomplished by drawing a vacuum in the vacuum chamber, using a standard laboratory pump. Thereafter, the filtrate is evaporated by heating the block under continued vacuum, and employing the vacuum blow-down principle of evaporation.

Finally, the dried oligonucleotide is resuspended in water and analyzed by HPLC using a gradient of 10-50% acetonitrile in 100 mmolar triethyl ammonium acetate over 20 minutes. The profile of the product profile on the resulting chromatogram (254 nm wavelength) shows that the DMT-DNA is eluted at about 13 minutes.

Example 6

Oligonucleotide Synthesis

A polyethylene depth filter is press fit within a standard polyethylene pipet tip. Granular controlled pore glass ("CPG", 1.5 mg) with 120 nmol per mg (Glen Research, Sterling, Virginia) is added to the top of the depth filter. A second, larger polyethylene depth filter is press fit into the pipet tip in order to retain the CPG within the tip. The outlet orifice of the tip is fitted with a female-female adaptor and the inlet orifice is provided in the form of a female luer connector, in order to fit a male luer fitting. These fittings are provided in order for the tip to be used on a commercially available DNA synthesizer.

A preprogrammed automated DNA synthesizer (BioSearch 8750) is used to synthesize a DMT-18-mer and is compared with a regular DNA synthesis column

synthesis of the same sequence. Analysis of the DMT-DNA on reversed phase HPLC shows the same product profile in both products. Using the filtration tip apparatus for DNA synthesis, however, provides significantly lower production costs as compared with regular columns.

Example 7

Filtration Tip and Solution Phase Reagents

Conventional DNA synthesis is performed in the manner described in Example 6 using a solid support material (CPG) contained within and/or between depth filters in a filtration tip, with cleavage and deprotection performed using aqueous reagents. The resulting DNA is purified by desalting and affinity chromatography on a separate hydrophobic resin.

This Example describes steps and reagents for use in the synthesis of oligonucleotides on solid support matrixes, including the processing of the oligonucleotide product (cleavage of the oligonucleotide from the support in aqueous ammonium hydroxide and deprotection of side chain protecting groups of the nucleobases A (Adenosine), C (Cytosine), G (Guanosine) in the DNA sequence in solution by aqueous ammonium hydroxide). T (Thymidine) has no side chain protection. The final oligonucleotide preparation is purified in a method based on the affinity of the DMT group for a hydrophobic resin.

Following affinity purification the DMT group is removed to provide a fully deprotected biologically compatible DNA. Desalting involves the removal of organics (e.g., protecting groups removed during deprotection) from the oligonucleotide preparation. Desalting can be performed by any suitable means, including by size exclusion chromatography or by precipitation of the oligonucleotide from alcohols.

The synthesis is performed using conventional techniques on an automated DNA synthesizer of the type commercially available from Applied Biosystems, PerSeptive Biosystems, Beckman Instruments, and others, with the column

attached using a suitable adaptor, e.g., a female to female luer adaptor. Such synthesizers are preprogrammed to deliver reagents and solvents for wash cycles through a column containing a solid support material, upon which the synthesis takes place. The solid support has been functionalized with nucleosides A, C, G or T. The nucleosides are typically attached to the support at their 3'-hydroxyl groups through an ester bond of succinic acid, which in turn is linked to the support through a succinyl amide linkage. A variety of commercially available supports are available, including polystyrene (Applied Biosystems, Pharmacia), CPG controlled pore glass (Glen Research, Cruachem, Beckman, PerSeptive Biosystems), PVDF membranes (PerSeptive Biosystems) to mention a few.

The nucleobases can be protected with a variety of protecting groups, ranging from those that are relatively labile to those that are relatively stable toward the reagents used in the deprotection of such protecting groups. Standard protecting groups include benzoyl protecting groups on C and A and isobutyryl group on G. More labile protecting groups can be used as well and include dimethylformamidine on G, acetyl on C, phenoxyacetyl on A, C and G.

The present Examples will describe the use of the standard protecting groups A^{Bz}, C^{Bz} and G^{IBu}. Cleavage of the ester bond attachment to the support is typically performed at room temperature using a concentrated ammonium hydroxide solution for 60 to 90 minutes, with deprotection of the protecting groups being performed in concentrated ammonium hydroxide for 8 hours at 55°C or 30 to 60 minutes at 85°C.

A typical DNA synthesis cycle is as follows: The 5'-DMT group of the nucleoside attached to the support is deprotected with acidic reagents, e.g., with 2% dichloracetic acid in dichloromethane, in order to expose the 5'-hydroxyl group for a coupling reaction with an activated 5'-DMT-nucleotide-3'-phosphoramidite in solution. The support is washed with acetonitrile and the DMT-monomer-nucleotide-phosphoramidite (in acetonitrile) is introduced into the column after activation with tetrazole in acetonitrile solution.

The formation of the internucleotide bond, phosphite triester, is almost instantaneous. The labile phosphite triester is oxidized with an iodine solution to form a stable phosphate triester. Most of the 5'-hydroxyl groups of the bound DNA are generally reacted with the amidite. Some 5-hydroxyl groups may not have reacted, however, for any number of reasons. Such unreacted 5'-hydroxyl groups if not inactivated, could react with the next nucleotide in the subsequent cycle and therefore generate an incorrect sequence that could interfere with the final product.

It is thus customary to introduce a "capping" step intended to inactive the unreacted 5'-hydroxyl groups and prevent their further reaction. Capping is commonly performed using acetic acid anhydride in the presence of a catalyst (N-Methyl-imidazole or dimethylaminopyridine). Once capping has been completed, the support is again washed and a new cycle started by the removal of the 5'-protecting group of the coupled nucleotide. By repeating these various steps a DNA oligomer is synthesized on the support.

In order to dissociate the bound oligonucleotide from the support the ester bond that attaches the 3'-hydroxyl group to the support is typically hydrolyzed (cleaved) at room temperature using aqueous ammonium hydroxide or aqueous methylamine. Since the process is typically performed in aqueous solution, the oligonucleotide (with side chain protecting groups still in place) is solubilized in the cleavage reagent. The side chain protecting groups need to be removed from the resulting solution in order to obtain the biologically active form of DNA.

Deprotection is typically performed by heating the solution at 55°C for a period of 8 hours to over-night when standard protecting groups are used. With more labile protecting groups the removal can be as fast as 5 to 10 minutes at 55°C. Using reagents provided in the gas phase, processing can be as fast as 2 minutes at room temperature for both the cleavage and deprotection steps, when methylamine is used to deprotect phenoxyacetyl type protecting groups. See, for instance, Nucleic Acids Research, 1996, Vol. 24, No. 15, 3115-3117.

Once the processing (cleavage and deprotection) of synthetic oligonucleotide is completed, the cleavage/deprotection reagent can be evaporated, and in certain applications, the oligonucleotide can be used without desalting and/or purification. In other applications, however, it is necessary or preferred that the oligonucleotide be desalted and/or provided in purified and fully deprotected form.

Desalting can be performed directly in the ammonium hydroxide solution by passing the solution through a size exclusion column (e.g., Nap column, Pharmacia Biotech). Optionally, the product can be desalted after evaporation of the cleavage/deprotection reagent by precipitating the oligonucleotide product from an alcohol.

Once the processing of synthetic DMT-oligonucleotide is completed, the oligonucleotide is preferably purified. Cartridge purification is faster and less expensive than HPLC chromatography. Standard protocols for cartridge purification are provided by the manufacturers. Cartridges typically contain a hydrophobic resin (commonly rigid polystyrene crosslinked with divinylbenzene) having a high affinity for the 5'-end protecting group of the synthetic DNA (DMT-group, dimethoxytrityl group).

A typical cartridge protocol (beginning with deprotected DMT-oligonucleotide in concentrated ammonium hydroxide) includes the following steps:

1. Dilute the stock solution with 2 volumes of 2 Molar triethylammonium acetate.

2. Condition the cartridge: Elute the cartridge with 1 ml of acetonitrile (wetting). Flush with air to remove excess. Then elute with 1 ml of 2 Molar triethylammonium acetate (increasing hydrophobicity of the support).

3. Apply the DMT-oligonucleotide solution, add 1 ml of the diluted stock solution (1) to the cartridge, two times with the syringe or pull back and forth from the stock solution.

4. Wash with 3% ammonium hydroxide (1 ml).

5. Wash the cartridge with 2 ml of water.

6. Detritylate with 2 ml of 2% TFA in water (trifluoroacetic acid).

7. Wash with 2 ml of water.

8. Elute the fully deprotected oligonucleotide in 1 ml of 20% aqueous acetonitrile.

9. Evaporate the oligo, redissolve in water, quantify and use.

Example 8

Gas Phase Reagents

A conventional DNA synthetic protocol is performed on a solid support (CPG and/or polystyrene) contained within and/or between depth filters in a filtration tip prepared in the manner described above, with cleavage and deprotection performed using reagents provided in the gas phase, and purification by desalting or affinity chromatography on a hydrophobic resin.

Oligonucleotide synthesis is performed in the manner set forth in Example 7. In columns having a combination support, of CPG and polystyrene, optionally only the CPG can be derivatized with the starting nucleoside. Cleavage and deprotection of the final product is performed using gaseous cleavage deprotection reagents, such as ammonia or methylamine, by removing the column from the synthesizer and placing it in a pressure chamber. Ammonia gas at 120 psi at room temperature or methylamine at 30 psi at room temperature is introduced under conditions (time, temperature, pressure) suitable to cleave and deprotect the oligonucleotide from the resin.

Optionally, gas phase processing can be performed with the filtration tip placed in an appropriate-sized polypropylene tube. The bottom of the tube contains an amount of aqueous ammonium hydroxide or methylamine that is much less than would be required for conventional solution chemistry, e.g., on the order of 0.2 ml, as opposed to 1 to 3 ml for solution phase processing. The tube is heated in a water-bath in order to degas the solution and generate an atmosphere

of gaseous reagent in the tube. The gaseous reagent serves to cleave the oligonucleotide from the support (cleavage of the ester bond) as well as deprotect the side chain protecting groups of the nucleotides.

The processing of the synthetic oligonucleotide is performed in the DNA synthesis column without the presence of water or solution-phase reagents that would otherwise elute the DNA from the support. The vapors are permitted to reach an equilibration state in which there is not sufficient condensation to elute the oligonucleotide from the resin in the column. As compared with the solution phase approach of Example 7, by virtue of gas phase processing of this Example the cleaved and deprotected oligonucleotides remain on the support. The DMT-oligonucleotide is retained in the column by its affinity for the hydrophobic filler and/or hydrophobic filter.

Desalting (removal of organic protecting groups) can be performed in the manner described above and within the original column used for synthesis. When the oligonucleotide is prepared on a support that includes polystyrene, and without the use of DMT-protecting groups, the support within the column can be washed with acetonitrile in order to remove the protecting groups that have been converted to amides (which are soluble in acetonitrile). Since the oligonucleotide is in the form of the ammonium salt, it will not be soluble in acetonitrile, and instead, can be separately eluted with water. The solution used to elute the oligonucleotide is neutral and the desalted oligonucleotide can be used without evaporation.

If, however, the oligonucleotide is prepared on a support that includes polystyrene and with a DMT group, the oligonucleotide can be purified by a shortened affinity chromatography procedure following gas phase cleavage and deprotection. Following synthesis and cleavage/deprotection the column can be desalted at the same time failure sequences are washed off the column, e.g., by the use of 3% ammonium hydroxide. Thereafter the remaining DMT-protected oligonucleotides can be detritylated by acid and eluted with water.

The gas phase processing, in combination with the support materials and columns of this invention provides for faster recovery, purification and desalting

of the oligonucleotide without the extra cost of size exclusion columns or purification cartridges.

Example 9

DMT Purification

Columns of this invention are prepared having a combination support material that includes hydrophilic DNA synthesis support (e.g., CPG) functionalized with nucleosides A, C, G and T in combination with an inert hydrophobic filler and/or filter material. Although in a preferred embodiment the filler does not itself serve as a support during synthesis, it does function in the course of affinity chromatography of DMT-oligos following synthesis on the CPG. Optionally, and preferably, the columns also provide one or more depth filters prepared from a suitable inert hydrophobic material (e.g., microfibrous depth filters of polyethylene, polypropylene, or PVDF) in order to enhance the hydrophobic content and function of the column.

In this manner, synthesis, cleavage and deprotection can all be performed within the column, employing the CPG component, while DMT purification can be performed in the same column using the inert hydrophobic material as an affinity chromatographic support.

A DNA synthesis column is prepared having a desired final capacity for DNA synthesis, by varying the capacity of the CPG component and the amount of the filler material accordingly. For instance, to achieve a capacity of 50 nmole, beginning with CPG having a capacity of 50 micromole/g (50 nmol per mg) such a column would require 1 mg of support. If, however, that CPG support is diluted 50% with polystyrene divinylbenzene (Amberchrom^{R}), then the combination support would require 2 mg total to contain the equivalent of 50 nmol. Along the same lines, if the CPG support is diluted 10-fold with polystyrene the total weight of the combination would requite 10 mg of mixture in order to contain 50 nmol of active CPG for synthesis.

Those skilled in the art will be able to determine the manner in which the actual amount of the combination support will vary, depending on the scale of synthesis to be performed, the initial functionalization of the CPG support, and so on. For example, CPG is typically functionalized at about 25 to 100 micromole per gram of support. Usually 500A pore size is used for DNA sequences shorter than 50 nucleotides and 1000A used for oligonucleotides of about 100 nucleotides in lengths. Extremely long oligonucleotides (150 or more nucleotides) benefit from CPG having a median pore size of about 2000A.

Hydrophobic materials useful as diluents in the present invention include synthetic, polymeric particulate supports capable of serving as an affinity support for DMT groups, without detrimental effect on the ability of the CPG component to serve as a support for oligonucleotide synthesis. An example of a preferred hydrophobic diluent is rigid cross-linked polystyrene divinylbenzene, such as that available from TosoHaas under the commercial name Amberchrom^{R}. This material is available in pore sizes of 300 A to 1000A and particle sizes between 30 to 150 microns. When the filler is to serve as affinity resin for DMT purification the dilution ratio is preferable 1:10 or more, (CPG to polystyrene, by weight). In situations in which affinity chromatography is not a concern, the dilution ratio can be on the order of 1:2 or less.

When CPG is used as a support for small scale synthesis (e.g., on the order of 1 nmol or less) the use of a hydrophobic filler performs a number of beneficial functions, in addition to its potential use as an affinity support. First, dilution of the hydrophilic support material with a hydrophobic filler permits the user to achieve a level of reproducability that is difficult to achieve by chemical means alone. Second, the ability of the hydrophobic filler to repel water permits more efficient washing and drying of the combination support. It is particularly important that traces of water be removed in order to avoid inactivating the expensive phosphoramidite used in synthesis.

Finally, when used in a filtration column of this invention, it is preferred that the combination support material be retained in the column by means of one

or more depth filters. In this case, the depth filter is desirably deeper than a surface filter or frit used in a conventional (e.g. CPG-containing) DNA synthesis column. The use of a depth filter not only facilitates the production of such columns, but also serves to further enhance hydrophobicity in the column.

In another embodiment, one or more hydrophobic depth filters can be used instead of, or in addition to, the hydrophobic particulate filler described above. Depth filters can be used to retain or disperse particulate DNA synthesis resin between or within the filters themselves. The presence of a hydrophobic depth filter in the column can contribute quite substantially to the hydrophobicity of the column. For instance, a microfibrous depth filter, formed of polyolefin and having a weight of about 15 mg, provides nearly a 100% increase of hydrophobic content. A column can thus be constructed to have one or more, and preferably two or more depth filters. Optionally, one or more depth filters can be compounded or coated with nonreactive hydrophobic additives, such as polysilicone and PVDF polymers, to further enhance their hydrophobic nature.

Using a combination support within a column of this invention, DNA synthesis, followed by either solution or gas phase cleavage and deprotection and standard purification are performed as described in Examples 7 or 8. The gas phase protocol of Example 8 provides added benefits by virtue of the fact that each step can be performed in the same column originally used for synthesis, including the use of hydrophilic CPG for synthesis and the hydrophobic polystyrene for affinity chromatography of DMT-oligos.

Example 10

Apparatus for Affinity Chromatography

A filtration column of this invention can be used as an affinity purification cartridge, for the purification of oligonucleotdes synthesized and recovered from another column, by providing the filtration column with one or more hydrophobic depth filters and/or particulate hydrophobic filler. In one embodiment, a column

is fitted with depth filters at each end of the column and filled with hydrophobic particulate resin between the depth filters.

Alternatively, more than two depth filters of hydrophobic material can be used in the column, in combination with particulate hydrophobic filler, or the entire column can be fitted with a single hydrophobic depth filter. Preferably, the filters are fibrous and can be compressed to fit the preferred conical shape of the apparatus. Both the diameter and the length of the filters can be varied in order to be press-fit in the apparatus of this invention. The filter density is on the order of 200 mg or more per cubic centimeter, and more preferably 400 mg or more per cubic centimeter when used as purification cartridge.

Solutions can be delivered to the column by any suitable means, e.g., by the use of a syringe with a male Luer fitting. Such a syringe can be used to deliver solutions at a higher back-pressure than that generally possible on a DNA synthesis column. The apparatus provides one female end for attachment to the syringe and an opposite end that optionally provides a male end for attachment to a manifold for multiple cartridge purifications.

Typically, 30 mg of hydrophobic support material (Amberchrom®, TosoHaas) is used, having a pore size of 300A and particle size of 50-100 microns. A depth filter is prepared of fibrous polyolefin, copolymer of polyethylene and polypropylene coated with a silane. When used for DMT cartridge purification the columns are optionally conditioned in the manner described above. The DMT-oligonucleotide in concentrated ammonium hydroxide is applied to the column, failure sequences are removed and the cartridge purification protocol is completed following the cartridge purification steps outlined above.

CLAIMS

What is claimed is:

1. An apparatus comprising a polymeric housing having a wall portion forming an internal passageway having a longitudinal axis, and a depth filter sealably positioned within the internal passageway.

2. An apparatus according to claim 1 wherein the housing wall provides an internal surface that defines an internal passageway comprising a conical portion.

3. An apparatus according to claim 2 wherein the opposing external and internal wall surfaces of the housing are parallel, and the apparatus is itself conical in shape.

4. An apparatus according to claim 1 wherein the wall itself is tapered along the length of the passageway, and toward the longitudinal axis, in order to form a generally conical tip.

5. An apparatus according to claim 1 wherein the tip provides a passageway having a shape selected from the group consisting of conical, cylindrical, barrel-shaped, stepped, toric, and frustroconical.

6. An apparatus according to claim 1 further comprising means for sealably attaching the passageway to another device.

7. An apparatus according to claim 6 wherein the other device is used to provide or withdraw materials and/or to provide positive or negative pressure at either or both ends of the passageway.

8. An apparatus according to claim 7 wherein the internal filter is selected from the group consisting of a single depth filter, a combination of a depth filter with one or more other filters, and a filter assembly comprising a particulate material positioned between two depth filters.

9. An apparatus according to claim 8 wherein the filter is a combination filter comprising a depth filter used to support a disc filter.

10. An apparatus according to claim 8 wherein the filter is a depth filter prepared from a material selected from the group consisting of porous polymeric resins, ion exchange resins, activated carbon, controlled pore glass, and combinations thereof.

11. An apparatus according to claim 8 wherein the filter is provided in the form of a filter assembly.

12. An apparatus according to claim 10 wherein the filter is prepared from a material selected from the group consisting of organic polymers, inorganic polymers, ceramics, and metals.

13. An apparatus according to claim 12 wherein the filter is prepared from an organic polymer selected from the group consisting of polyurethanes, polyacrylonitrile, polyvinyl alcohol, poyvinyl acetate, polyester, polyamide, polystyrene, polysulfone, cellulose, cellulose acetate, polyethylene, polypropylene, polyvinyl fluoride, polyvinylidene fluoride, polytrifluorochloroethylene, polyvinylidene fluoride-tetrafluoroethylene copolymer, polyethersulfone, poly(meth)acrylate, butadiene-acrylonitrile copolymer, polyether-polyamide block copolymer, and ethylene-vinyl alcohol copolymer.

14. A system for processing solutions, the system comprising:

(a) a vacuum chamber dimensioned to enclose one or more receptacle sites, the chamber comprising a cover portion comprising one or more apertures, each in an overlapping position with respect to a corresponding receptacle site,

(b) one or more filtration tips according to claim 1, each tip being sealably positioned within a respective cover aperture, with its outlet passageway directed toward a respective receptacle site, and

(c) a vacuum system for controllably creating a vacuum within the chamber in a manner sufficient to draw solution from the inlet passageway of a filtration tip, through the filter and into a corresponding receptacle site.

15. A method of processing a sample, the method comprising the steps of:

(a) providing a system comprising a vacuum chamber and one or more filtration tips according to claim 1;

(b) delivering solution to the inlet passageway of the filtration tip(s);

(c) optionally, using the inlet passageway as an incubation vessel for the solution;

(d) creating a vacuum in the chamber in order to draw the solution into the filter;

(e) optionally, using the filter as an incubation vessel for the solution;

(f) maintaining a vacuum in the chamber in order to draw the solution through the filter and into a solution receptacle site, and optionally;

(g) employing the vacuum to create a vacuum blow down effect in order to evaporate the filtrate.

16. A method according to claim 15 wherein the method is used for the synthesis and recovery of an oligonucleotide.

17. An apparatus comprising a polymeric housing having a wall portion forming an internal passageway having a longitudinal axis, and an internal filter sealably positioned within the internal passageway, wherein the internal filter is selected from the group consisting of a single depth filter, a combination of a depth filter with one or more other filters, and a filter assembly comprising particulate material positioned within and/or between a plurality of depth filters, wherein the filter comprises a hydrophobic support material.

18. An apparatus according to claim 17 wherein the filter comprises particulate controlled pore glass support in combination with a particulate hydrophobic polystyrene filler material, retained in the column by the use of one or more depth filters.

19. An apparatus according to claim 18 wherein only the CPG support material is derivatized with an initial nucleoside.

20. A method of preparing synthetic oligonucleotides, the method comprising the steps of:

(a) synthesizing DMT-protected oligonucleotides on a support material that comprises a hydrophobic component,

(b) cleaving and deprotecting the oligonucleotides from the support material by the use of an amine reagent provided in the gas phase, and removing the reagent under conditions suitable to permit the cleaved, deprotected, DMT-protected oligonucleotides to remain on the support and bound to the hydrophobic component,

(c) purifying the oligonucleotides by removing failure sequences by the use of a solvent having suitable properties to permit the DMT-protected oligonucleotides to remain bound to the support while solubilizing oligonucleotides not containing a DMT-protecting group,

(d) fully deprotecting the bound DMT-protected oligonucleotides by detritylating with a suitable reagent, and

(e) eluting the cleaved, fully deprotected, purified and detritylated oligonucleotide from the column.

21. A method according to claim 20 wherein cleavage and deprotection is performed using a gas selected from the group consisting of gaseous ammonia, gaseous methyl amine, ammonium hydroxide vapors, and mixtures thereof, failure sequences are removed by the use of 1% to 5% ammonium hydroxide, the detritylating step is performed by the use of 1% to 5% trifluoroacetic acid and the eluting step is performed by the use of an aqueous buffer or water.

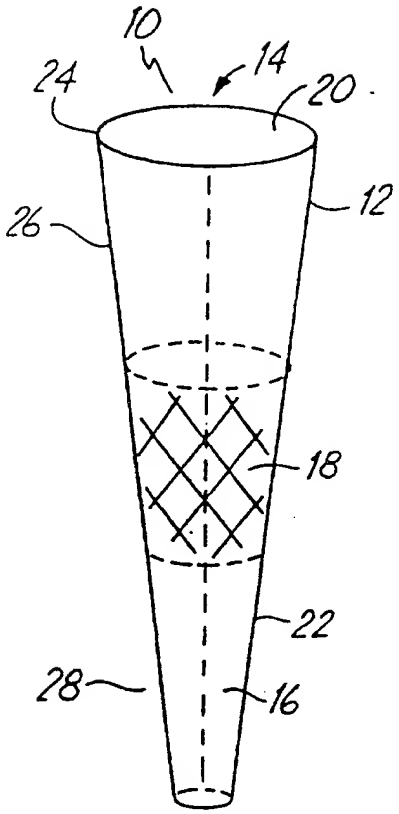

Fig. 1

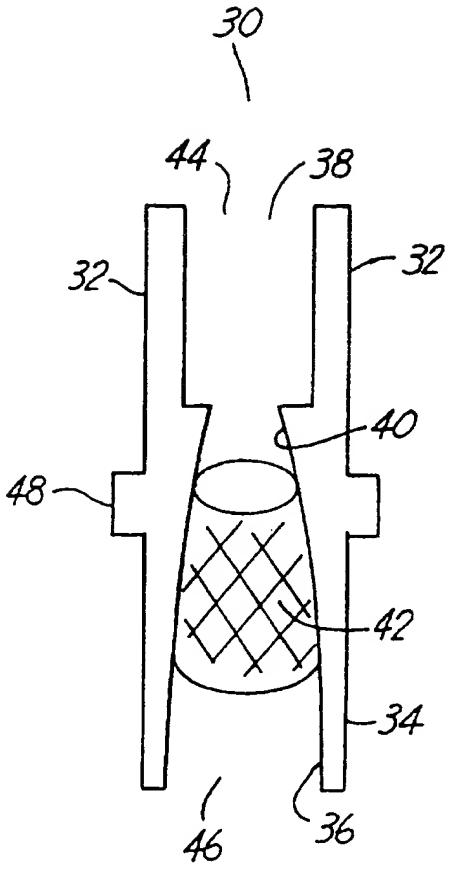

Fig. 2

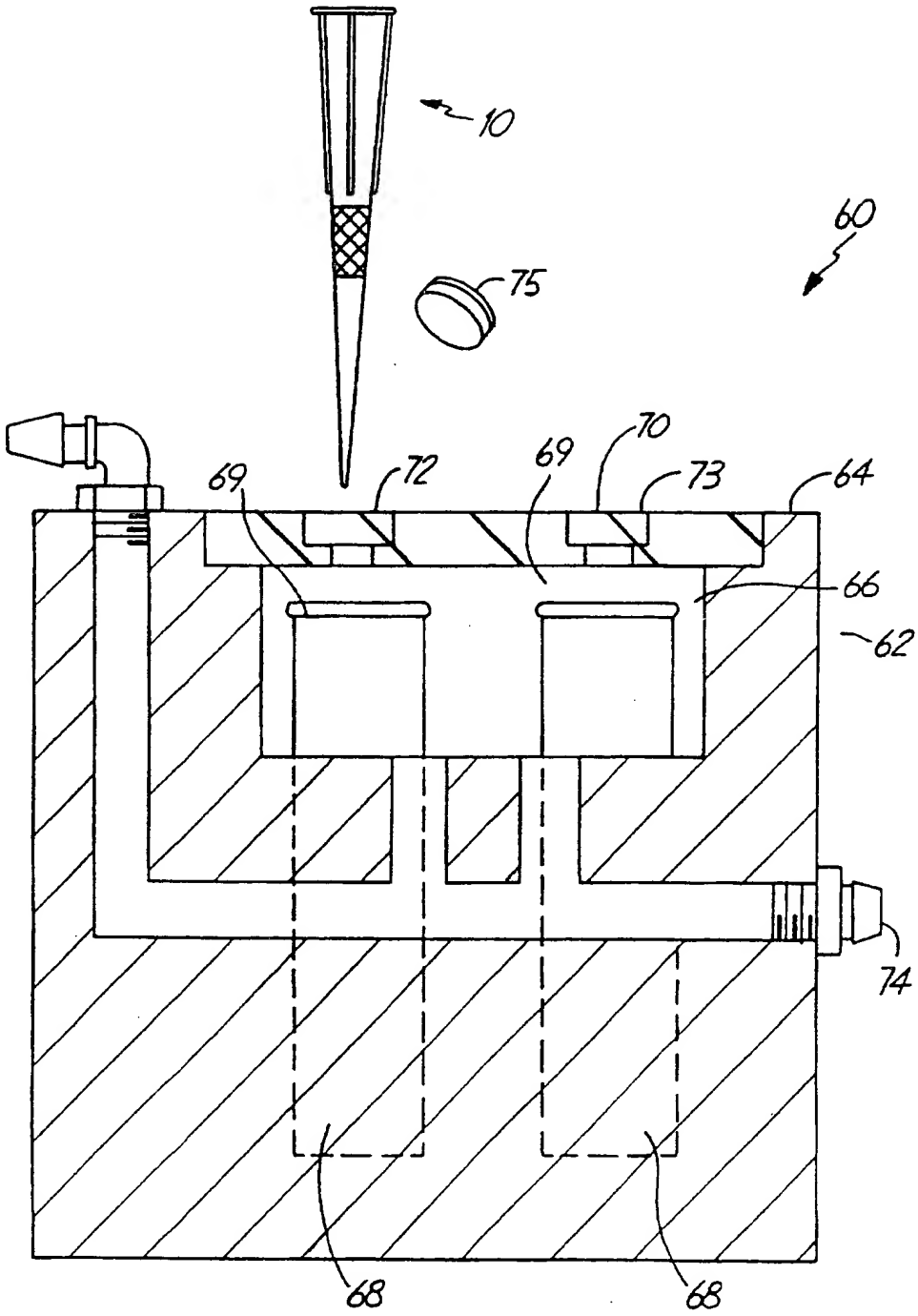

Fig. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/00441

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 35/00; C12Q 1/68
US CL :436/178
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/177, 178, 180; 422/100, 101, 68.1; 73/863.23, 863.32, 864.11; 210/406, 416.1, 477, 500.36, 500.42; 435/6, 289

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,156,811 A (WHITE) 20 October 1992, see entire document.	1-5,13-14
X — Y	US 5,364,595 A (SMITH) 15 November 1994, see entire document.	1-7, 13-14 — 8-12, 15-21
X — Y	US 5,437,979 A (RAMPAL et al) 01 August 1995, see entire document.	1-14,17-19 — 15,16,20-21
A	PON et al., Derivatization of Controlled Pore Glass Beads for Solid Phase Oligonucleotide Synthesis, 1988, Vol 6, No. 8, pages 768-775.	15, 16, 20, 21

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 APRIL 1997	30 APR 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer HAROLD Y. PYON Telephone No. (703) 308-0651

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/00441

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JAGADEESWARAN, Automated Column Equilibration, Washing, Sample Loading and Elution of Bench-Packed Mini-Co lumns, BioTechniques, Vol 12, No. 3, 1992, pages 336-340.	15, 16, 20, 21
A	SCHOTT, A simple manual method for oligonucleotide synthesis, Amer. Biotech. Lab., January/February 1985, pages 20-23.	15-16, 20-21
A	JP 57-35707 (FUJITA et al) 31 July 1982, see entire document.	1-21